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# Interaction of sulphonylurea derivatives with vascular ATP-sensitive potassium channels in humans

**P.J. Bijlstra[1], J.A. Lutterman[1], F.G.M. Russel[2], T. Thien[1], P. Smits[1,2]**

[1] Department of Internal Medicine, University Hospital Nijmegen, Nijmegen, The Netherlands
[2] Department of Pharmacology, University of Nijmegen, Nijmegen, The Netherlands

**Summary** Cardiovascular adenosine-5′-triphosphate-sensitive potassium ($K_{ATP}$) channels have been reported to play an important role in endogenous cardioprotective mechanisms. Sulphonylurea derivatives can inhibit these cardioprotective mechanisms in animal models. We investigated whether therapeutic concentrations of sulphonylurea derivatives can block vascular $K_{ATP}$ channels in humans. The forearm vasodilator responses to administration of the specific $K_{ATP}$ channel opener diazoxide into the brachial artery of healthy male volunteers were recorded by venous occlusion plethysmography. This procedure was repeated with concomitant intraarterial infusion of: 1) the sulphonylurea derivative glibenclamide (0.33 or 3.3 $\mu g \cdot min^{-1} \cdot dl^{-1}$, both $n = 12$), 2) the new sulphonylurea derivative glimepiride (2.5 $\mu g \cdot min^{-1} \cdot dl^{-1}$, $n = 12$) or 3) placebo ($n = 12$). The effects of glibenclamide on the vasodilator responses to sodium nitroprusside were also studied ($n = 12$). Glibenclamide significantly inhibited the diazoxide-induced increase in forearm blood flow ratio (ANOVA with repeated measures: $p < 0.01$). During the highest diazoxide dose this ratio (mean ± SEM) was lowered from 892 ± 165 to 449 ± 105 %, and from 1044 ± 248 to 663 ± 114 % by low- and high-dose glibenclamide, respectively. In contrast, neither glimepiride nor placebo attenuate diazoxide-induced vasodilation. Furthermore, glibenclamide did not affect nitroprusside-induced vasodilation. We conclude that therapeutic concentrations of the classical sulphonylurea derivative glibenclamide result in significant blockade of vascular $K_{ATP}$ channels in humans. The newly developed glimepiride seems to be devoid of these properties. [Diabetologia (1996) 39: 1083–1090]

**Keywords** Glibenclamide, glimepiride, diazoxide, forearm blood flow, cardioprotection, sulphonylurea derivatives, potassium channels, vascular.

Sulphonylurea (SU) derivatives have represented the backbone of non-insulin-dependent diabetes mellitus (NIDDM) therapy for several decades [1]. These drugs exert their primary effect by closing so-called adenosine-5′-triphosphate-sensitive potassium ($K_{ATP}$) channels in the beta cell of the pancreas, which promotes an influx of calcium with subsequent stimulation of insulin release [2]. Recent investigations have shown that the cardiovascular system also shares functional $K_{ATP}$ channels [3]. Under physiological circumstances these channels are closed or inactive [4]. During hypoxia and/or ischaemia, the intracellular concentration of ATP falls which is a direct signal for $K_{ATP}$ channels to open. The subsequent efflux of potassium and hyperpolarization of the cell membrane induces a shortening of the action potential in the myocardium and a relaxation of the vascular smooth muscle cell [5, 6]. Animal experiments have shown that these mechanisms play a role in the protection of the myocardium against ischaemia and reperfusion damage [7].

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*Corresponding author:* Professor P. Smits, Department of Pharmacology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

*Abbreviations*: $K_{ATP}$ channel, Adenosine-5′-triphosphate-sensitive potassium channel; SU, sulphonylurea; FBF, forearm blood flow; SNP, sodium nitroprusside; FVR, forearm vascular resistance; MAP, mean arterial pressure; NIDDM, non-insulin-dependent diabetes mellitus.

**Table 1.** Characteristics of the healthy volunteers

| | Pilot | Glibenclamide low-dose | Glibenclamide high-dose | Glimepiride | Time control | Vasodilator control |
|---|---|---|---|---|---|---|
| *n* | 4 | 12 | 12 | 12 | 12 | 12 |
| Age (years) | 24 ± 5 | 27 ± 9 | 27 ± 7 | 30 ± 11 | 33 ± 9 | 30 ± 9 |
| Weight (kg) | 67 ± 6 | 75 ± 9 | 76 ± 7 | 79 ± 8 | 81 ± 12 | 75 ± 11 |
| Height (m) | 1.80 ± 0.07 | 1.79 ± 0.06 | 1.81 ± 0.07 | 1.81 ± 0.06 | 1.82 ± 0.07 | 1.84 ± 0.06 |
| Body mass index (kg/m$^2$) | 22 ± 2 | 23 ± 2 | 23 ± 2 | 24 ± 2 | 24 ± 2 | 22 ± 3 |
| Systolic blood pressure (mm Hg)[a] | 124 ± 3 | 127 ± 9 | 125 ± 6 | 128 ± 7 | 130 ± 8 | 128 ± 4 |
| Diastolic blood pressure (mm Hg)[a] | 71 ± 6 | 67 ± 9 | 69 ± 10 | 74 ± 11 | 71 ± 11 | 75 ± 6 |
| Heart rate (bpm) | 68 ± 3 | 64 ± 4 | 65 ± 7 | 62 ± 5 | 64 ± 7 | 67 ± 8 |

Data are presented as means (± SD). [a] Sphygmomanometrically obtained blood pressure after 5 min supine rest

Interestingly, vascular $K_{ATP}$ channels can be activated pharmacologically by $K_{ATP}$ channel-opening drugs such as diazoxide, pinacidil, cromakalim and bimakalim [4]. These drugs have been shown to interact directly with the $K_{ATP}$ channel without interfering with the SU receptor [8]. Hence, [$^3$H]glibenclamide binding to the solubilized SU receptor was not inhibited by diazoxide [9], whereas glibenclamide binding to SU receptors of intact cells can indirectly be inhibited by $K_{ATP}$ channel openers [10]. In animal experiments, glibenclamide has been shown to inhibit the vasodilator as well as the cardioprotective responses to $K_{ATP}$ channel-opening drugs [7, 11]. Moreover, SU derivatives have been demonstrated to attenuate the vasodilator response to an ischaemic stimulus [12] and to abolish the protective effects of myocardial ischaemic preconditioning [13, 14]. In theory, the aforementioned observations may implicate harmful cardiovascular effects of SU derivatives when used under conditions of ischaemia in patients with NIDDM. However, up to now no data in humans on the cardiovascular effects of SU derivatives at clinically relevant concentrations are available.

Therefore, we investigated the ability of the SU derivative glibenclamide to reduce the vasodilator response to $K_{ATP}$ channel-activation in man. Further, we included studies with the newly developed SU derivative glimepiride which has been shown to be devoid of vascular $K_{ATP}$ channel binding properties in animal models [15, 16].

## Subjects and methods

The aim of this study was to investigate the putative inhibitory effects of glibenclamide on the $K_{ATP}$ channel-mediated vasodilation by diazoxide. In order to address the hypothesis of our study, the perfused forearm technique was used [17]. The study protocol was approved by the local ethics committee, and all participants gave written informed consent before entering the study. All experiments were performed in healthy male non-smoking volunteers with a normal medical history, physical examination and blood pressure. The characteristics of the subsets of volunteers are listed in Table 1. Each volunteer participated in only one experiment and was instructed to abstain from caffeine-containing beverages and alcohol at least 24 h before the experiment. Furthermore, they were asked to eat a light meal at 2 h before the experiment was started and to abstain from further food intake until after the experiment. Forearm volume was measured by water displacement. The experiments were performed with the subjects in the supine position in a quiet temperature controlled room (22 °C) to ensure that forearm blood flow (FBF) predominantly referred to forearm muscle perfusion [18].

*Glibenclamide study.* The effects of glibenclamide on the diazoxide-mediated vasodilation were studied. A cannula was inserted into the brachial artery (Angiocath, 20 gauge; Deseret Medical Inc., Becton Dickinson, Sandy, Utah, USA). For some of the protocols an antecubital vein of the left and the right arm was also cannulated. After an equilibration period of 45 min, a 35 min intraarterial infusion of placebo (154 mmol/l NaCl) was started. After 10 min, baseline measurements of blood pressure, heart rate and bilateral FBF were taken during the concomitant intraarterial placebo infusion. Then, a dose-response curve of the vasodilator effects of diazoxide was made (three intraarterial dosages: 0.25, 0.75, 2.25 mg · min$^{-1}$ per dl of forearm volume, 5 min per dose). All drugs were infused at a volume rate of 50 µl · min$^{-1}$ · dl$^{-1}$ using an automated syringe infusion pump (type STC-521; Terumo Corp., Tokyo, Japan). Blood pressure was measured via the intraarterial line using a Hewlett Packard monitor (type 78353B; Böblingen, Germany). FBF was measured by ECG-triggered venous occlusion mercury-in-silastic strain-gauge plethysmography (Hokanson EC4; D.E. Hokanson, Inc., Issaquah, Wash., USA). During all recordings of the FBF, the hand circulation was completely occluded by a wrist cuff inflated 100 mmHg above the systolic blood pressure to be sure that measurements only referred to the forearm skeletal muscle vascular bed [19]. After a subsequent equilibration of 60 min to allow parameters to return to baseline levels, baseline values during concomitant intraarterial infusion of placebo were again recorded. Then a second dose-response curve was made in 12 subjects, but with concomitant infusion into the brachial artery of glibenclamide instead of placebo.

In a pilot study with four volunteers, we infused five dosages of glibenclamide (0.1, 0.33, 1.0, 3.3, 10.0 µg · min$^{-1}$ · dl$^{-1}$, 10 min per dose) into the brachial artery, and sampled blood from the neighbouring vein to determine which infusion rate would lead to regional serum concentrations of about 200 ng/ml as maximally occurs after oral administration of 5 mg glibenclamide to diabetic patients [20, 21]. These five infusions resulted in regional concentrations of 43 ± 15, 138 ± 39, 564 ± 143, 1926 ± 363 and 5141 ± 1207 ng/ml, respectively. The second dose of glibenclamide (0.33 µg · min$^{-1}$ · dl$^{-1}$) was chosen for the final experiments. In order to investigate possible dose-dependent effects of glibenclamide on the diazoxide-mediated vasodilation, we also used a dose of glibenclamide 10 times higher (3.3 µg · min$^{-1}$ · dl$^{-1}$) in another 12 subjects.

Venous blood samples from the non-experimental arm were taken for insulin and C-peptide as well as for glibenclamide determinations before and at the end of glibenclamide infusion. After each diazoxide infusion venous blood samples from the experimental side were taken for the determination of local glibenclamide concentrations. At all these time points the plasma glucose concentration was also measured. Glucose was measured immediately by an Accutrend glucose analyzer (type 1284851; Boehringer, Mannheim, Germany).

Because of the long half-life, high protein binding [20, 21] and possible incorporation of SU derivatives in the plasma membrane [22], randomization of placebo and SU derivative was not possible because of carryover effects.

*Glimepiride study.* Apart from glibenclamide, we also studied the newly developed SU derivative glimepiride. Glimepiride strongly resembles glibenclamide on a molecular basis [23], but pharmacodynamically they have to be distinguished. Like the other SU derivatives, glimepiride also stimulates insulin secretion from the beta cell of the pancreas [24], but glimepiride and glibenclamide have different binding sites in the pancreatic beta cell [25, 26]. Furthermore, in contrast to glibenclamide, animal models have not shown any interaction of glimepiride with vascular $K_{ATP}$ channels [15, 16].

To investigate whether glimepiride is devoid of inhibitory effects on the $K_{ATP}$ channel-mediated vasodilation by diazoxide, the above-described protocol was performed with glimepiride instead of glibenclamide ($n = 12$). Maximal concentrations after regular once daily oral administration of 4 mg glimepiride to NIDDM patients were about 300 ng/ml. In our experiments we used a dose of 2.5 $\mu g \cdot min^{-1} \cdot dl^{-1}$ of glimepiride, which is thought to result in regional concentrations above the therapeutic range as seen during treatment of NIDDM [23, 24, 27].

*Time control study.* In order to exclude any influence of time or the repeat of the diazoxide infusions, a time-control study was performed in another 12 subjects. This study was identical to the previous studies, except that placebo was *not* replaced in the second part, resulting in two dose-response curves of diazoxide with concomitant intraarterial infusion of placebo.

*Vasodilator control study.* To show that glibenclamide had specific effects on $K_{ATP}$ channel-mediated vasodilation, we investigated the effects of glibenclamide on the vasodilator response to sodium nitroprusside (SNP) in another 12 subjects. This part was identical with the low-dose glibenclamide study, except that the three intraarterial diazoxide dosages were replaced by three intraarterial dosages of SNP (0.06, 0.2, 0.6 $\mu g \cdot min^{-1} \cdot dl^{-1}$, 5 min per dose). In this series, glibenclamide serum concentrations as well as glucose, insulin and C-peptide concentrations were determined before and after glibenclamide infusion.

*Humoral parameters.* For glibenclamide and glimepiride determinations venous blood samples were collected in glass tubes without additives. After 20 min the blood was centrifuged at 3000 routes/min for 10 min. Then serum was frozen at –20 °C. In these serum samples drug concentrations were determined at the laboratories of Hoechst AG, Frankfurt, Germany. Glibenclamide was determined, using a validated specific radioimmunoassay (RIA) [28]. The detection limit was 1–3 ng/ml and concentrations higher than 200 ng/ml were diluted before being measured according to the standard procedure. Glimepiride was determined by high performance liquid chromatography (HPLC) [29]. The limit of quantification was 10 ng/ml, and the detection limit was 5 ng/ml.

Insulin and C-peptide were determined in venous blood samples collected in chilled glass tubes coated with lithium-heparin. The blood was centrifuged at 3000 routes/min for 10 min. Then plasma was frozen at –20 °C. In these samples insulin and C-peptide were determined in our laboratories using specific RIAs. C-peptide was measured with a standard kit (D.P.C., Los Angeles, Calif., USA) and insulin with a procedure using standard and tracer prepared from monocomponent human insulin (NOVO, Zoeterwoude, The Netherlands).

*Calculations.* Forearm vascular resistance (FVR) was calculated as the quotient of mean arterial pressure (MAP) and FBF. The ratio of experimental to non-experimental FBF was calculated to correct for systemic changes due to time or arousal in order to refer only to changes induced by local infusions [30]. For each dose of diazoxide and SNP, FBF registrations of the last 2 min of infusion were averaged to one mean representative value. Likewise, absolute and percentage changes from baseline were calculated. In the same manner values for FVR and FBF ratio were obtained.

### *Statistical analysis*

The results were analysed statistically by an analysis of variance (ANOVA) with repeated measures over all vasodilator dosages. A $p$-value of less than 0.05 was considered statistically significant. Because of significant differences in baseline values this analysis was performed on the percentage changes from baseline for the FBF ratio, but also for the FBF and FVR, followed by post-hoc $t$-tests when ANOVA was significant. Values presented are means ± SEM unless indicated otherwise.

## Results

*Vascular effects of diazoxide.* To characterize the diazoxide-induced changes, the data of 48 subjects in which the first dose-response curve of diazoxide was performed were pooled. In Table 2 the effects of diazoxide infusions into the brachial artery on FBF, FVR, FBF ratio, MAP and heart rate are shown. Diazoxide infusions induced a dose-dependent forearm vasodilator response. The FBF and FVR on the non-experimental side remained unchanged. Diazoxide-induced changes in MAP and heart rate were small but statistically significant and occurred only during the highest dose. In 12 subjects a time-control study was performed. From baseline values (1.2 ± 0.1), FBF ratio increased 1.4 ± 0.3, 2.8 ± 0.6 and 6.3 ± 1.4 during a first dose-response curve of diazoxide. After 1 h of equilibration, FBF ratio had returned towards baseline levels (1.7 ± 0.2) after which it increased 1.5 ± 0.3, 2.5 ± 0.5 and 7.6 ± 1.5 during a second dose-response curve. These absolute changes as well as the percentage changes from baseline during the first and second dose-response curves showed no significant differences ($p = 0.63$ and $p = 0.51$ respectively).

*Vascular effects of sulphonylurea derivatives.* Ten minutes of the single intraarterial infusion of glibenclamide

Table 2. Pooled data of 48 subjects at baseline and during diazoxide with placebo infusions

| $n = 48$ | Baseline 1 | Diazoxide 0.25 | Diazoxide 0.75 | Diazoxide 2.25 | Baseline 2 |
|---|---|---|---|---|---|
| FBF-experimental (ml · min$^{-1}$ · dl$^{-1}$)[a] | 1.9 ± 0.1 | 4.0 ± 0.2[b] | 6.7 ± 0.4[b] | 14.2 ± 1.0[b] | 3.0 ± 0.2[b] |
| FBF-control (ml · min$^{-1}$ · dl$^{-1}$) | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1 | 1.7 ± 0.1 |
| FBF ratio[a] | 1.3 ± 0.1 | 2.9 ± 0.2[b] | 4.8 ± 0.4[b] | 11.6 ± 1.3[b] | 1.9 ± 0.1[b] |
| FVR-experimental (AU)[a] | 51.7 ± 2.7 | 25.2 ± 1.6[b] | 16.3 ± 1.3[b] | 8.0 ± 0.9[b] | 37.6 ± 2.6 |
| FVR-control (AU) | 63.5 ± 5.1 | 66.9 ± 5.7 | 63.9 ± 5.8 | 69.9 ± 6.7 | 62.9 ± 4.5 |
| MAP (mm Hg)[a] | 85 ± 1 | 85 ± 1 | 84 ± 1 | 82 ± 1[b] | 86 ± 1 |
| Heart rate (bpm)[a] | 58 ± 1 | 59 ± 1 | 60 ± 1 | 65 ± 2[b] | 59 ± 1 |

Data are mean ± SEM. Forearm blood flow (FBF) and forearm vascular resistance (FVR in arbitrary units, AU), both at the experimental and non-experimental control side, and mean arterial pressure (MAP) and heart rate (in beats per minute, bpm). [a] Repeated measures ANOVA over three diazoxide dosages $p < 0.05$; [b] Bonferroni corrected $t$-test vs baseline 1 $p < 0.005$

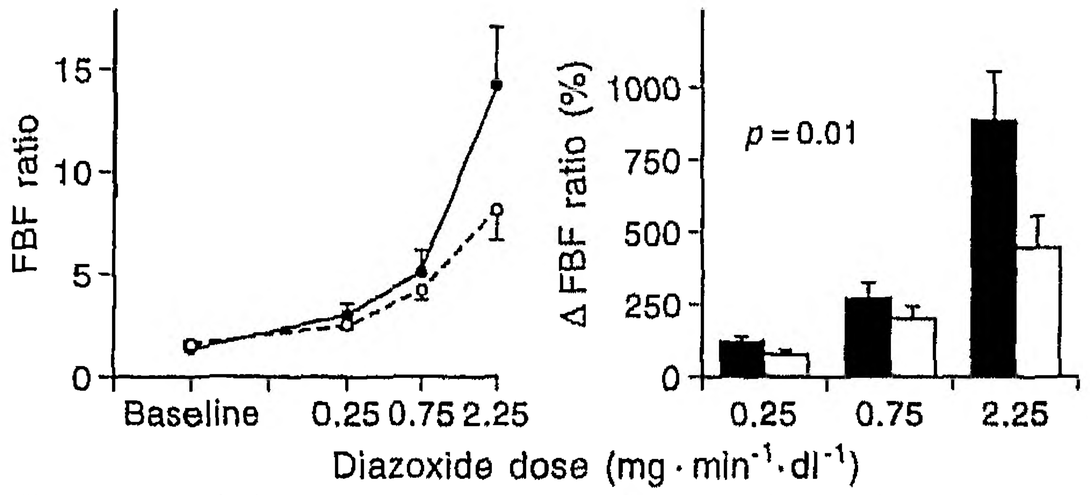


**Fig. 1.** The left panel shows mean (± SEM) absolute FBF ratio's at baseline and during intraarterial infusion of the three diazoxide dosages (0.25, 0.75, 2.25) with concomitant intraarterial infusion of placebo (●), or with concomitant intraarterial infusion of low-dose glibenclamide (○). The right panel shows the corresponding mean (± SEM) percentage changes from baseline in FBF ratio. (■) Placebo; (□) low-dose glibenclamide. The $p$-value refers to ANOVA with repeated measures over the complete dose-response curves

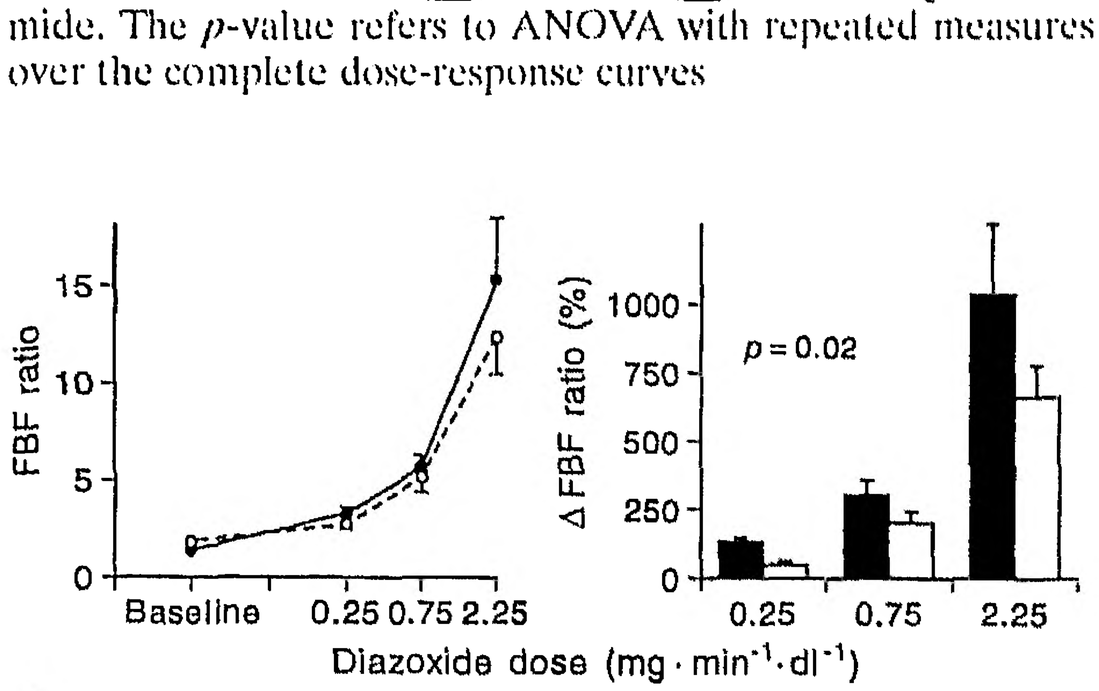


**Fig. 2.** The left panel shows mean (± SEM) absolute FBF ratio's at baseline and during intraarterial infusion of the three diazoxide dosages (0.25, 0.75, 2.25) with concomitant intraarterial infusion of placebo (●), or with concomitant intraarterial infusion of high-dose glibenclamide (○). The right panel shows the corresponding mean (± SEM) percentage changes from baseline in FBF ratio. (■) Placebo; (□) high-dose glibenclamide. The $p$-value refers to ANOVA with repeated measures over the complete dose-response curves

alone (0.33 μg · min$^{-1}$ · dl$^{-1}$) did not alter the FBF ratio (change: 0.02 ± 0.08). This number did not differ statistically from the change induced by placebo in the time-control series (0.04 ± 0.26). The same was true for the high-dose glibenclamide and the glimepiride series. We did not observe any effect of the SU derivatives on blood pressure and heart rate.

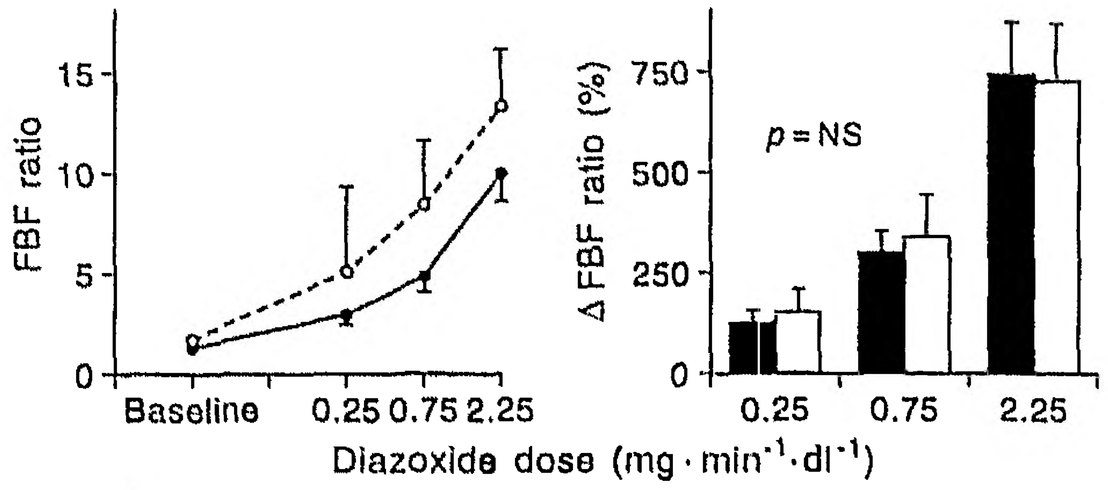


**Fig. 3.** The left panel shows mean (± SEM) absolute FBF ratio's at baseline and during intraarterial infusion of the three diazoxide dosages (0.25, 0.75, 2.25) with concomitant intraarterial infusion of placebo (●), or with concomitant intraarterial infusion of high-dose glimepiride (○). The right panel shows the corresponding mean (± SEM) percentage changes from baseline in FBF ratio. (■) Placebo; (□) glibenclamide. The $p$-value refers to ANOVA with repeated measures over the complete dose-response curves. NS, not significant

*Effects of SU derivatives on vasodilator responses.* In Figure 1 and 2 it is clearly seen that absolute values of the FBF ratio (left panels) and its percentage changes from baseline (right panels) in response to diazoxide infusions were lower during co-infusion of glibenclamide than during co-infusion of placebo (low-dose $p = 0.01$, high-dose $p = 0.02$), indicating a statistically significant reduction of the vasodilator responses to diazoxide during glibenclamide infusion. In Table 3 this is also shown for the changes in FBF and FVR. The reductions in diazoxide-mediated vasodilation by low- and high-dose glibenclamide were not significantly different from each other ($p = 0.98$). Post-hoc $t$-tests showed a significant inhibition of vasodilation by low-dose glibenclamide induced only during the highest diazoxide dose ($p = 0.008$), whereas the high-dose glibenclamide significantly inhibited the lowest ($p < 0.001$) and the middle ($p = 0.04$) diazoxide dose.

Figure 3 shows that, in contrast to glibenclamide, the new SU derivative glimepiride had no inhibitory effects on the vasodilator responses to diazoxide. Figure 4 shows that low-dose glibenclamide did not affect the vasodilator responses to the $K_{ATP}$ channel independent vasodilator SNP.

Similar to the first series of diazoxide infusions (Table 2), slight changes in MAP and heart rate

**Table 3.** Values with changes during diazoxide with concomitant placebo or (low- or high-dose) glibenclamide infusion

| | Low-dose Glibenclamide | | | | High-dose Glibenclamide | | | |
|---|---|---|---|---|---|---|---|---|
| | Forearm blood flow | | Forearm vascular resistance | | Forearm blood flow | | Forearm vascular resistance | |
| Baseline 1 (placebo) | 1.6 ± 0.1 (ml · min$^{-1}$ · dl$^{-1}$) | | 57.5 ± 5.3 (AU) | | 2.1 ± 0.2 (ml · min$^{-1}$ · dl$^{-1}$) | | 44.9 ± 3.0 (AU) | |
| *Changes from baseline* | (ml · min$^{-1}$ · dl$^{-1}$) | (%) | (AU) | (%) | (ml · min$^{-1}$ · dl$^{-1}$) | (%) | (AU) | (%) |
| Diazoxide 0.25 (placebo) | 1.8 ± 0.3 | 117 ± 22 | −29.6 ± 4.8 | −49.8 ± 4.8 | 2.4 ± 0.3 | 120 ± 14 | −24.4 ± 2.4 | −53.3 ± 3.3 |
| Diazoxide 0.75 (placebo) | 4.2 ± 0.8 | 282 ± 58 | −39.4 ± 5.9 | −66.3 ± 5.9 | 5.4 ± 0.8 | 272 ± 43 | −32.2 ± 2.3 | −71.7 ± 2.2 |
| Diazoxide 2.25 (placebo) | 12.3 ± 2.3 | 771 ± 154 | −46.2 ± 6.6 | −81.9 ± 5.7 | 13.9 ± 1.8 | 691 ± 100 | −38.8 ± 2.5 | −86.5 ± 1.3 |
| Baseline 2 (placebo) | 2.6 ± 0.4 (ml · min$^{-1}$ · dl$^{-1}$) | | 41.9 ± 5.6 (AU) | | 2.7 ± 0.3 (ml · min$^{-1}$ · dl$^{-1}$) | | 39.1 ± 5.0 (AU) | |
| Baseline 3 (glibenclamide) | 2.6 ± 0.5 (ml · min$^{-1}$ · dl$^{-1}$) | | 45.3 ± 6.7 (AU) | | 2.4 ± 0.3 (ml · min$^{-1}$ · dl$^{-1}$) | | 44.3 ± 5.7 (AU) | |
| *Changes from baseline* | (ml · min$^{-1}$ · dl$^{-1}$) | (%) | (AU) | (%) | (ml · min$^{-1}$ · dl$^{-1}$) | (%) | (AU) | (%) |
| Diazoxide 0.25 (glibenclamide) | 1.3 ± 0.2 | 62 ± 13 | −17.7 ± 4.9 | −34.5 ± 5.1 | 1.8 ± 0.2 | 78 ± 10 | −20.3 ± 3.7 | −43.9 ± 2.8 |
| Diazoxide 0.75 (glibenclamide) | 3.4 ± 0.9 | 164 ± 42 | −23.2 ± 7.4 | −44.8 ± 14.0 | 4.7 ± 0.7 | 213 ± 34 | −30.2 ± 4.7 | −66.3 ± 3.5 |
| Diazoxide 2.25 (glibenclamide) | 8.7 ± 2.4 | 372 ± 97 | −29.6 ± 5.3 | −69.2 ± 6.4 | 12.4 ± 1.6 | 584 ± 94 | −37.7 ± 5.3 | −83.9 ± 1.7 |
| $p$-value from ANOVA with repeated measures over all diazoxide dosages | | 0.01 | | 0.009 | | 0.03 | | 0.02 |

Values are mean ± SEM

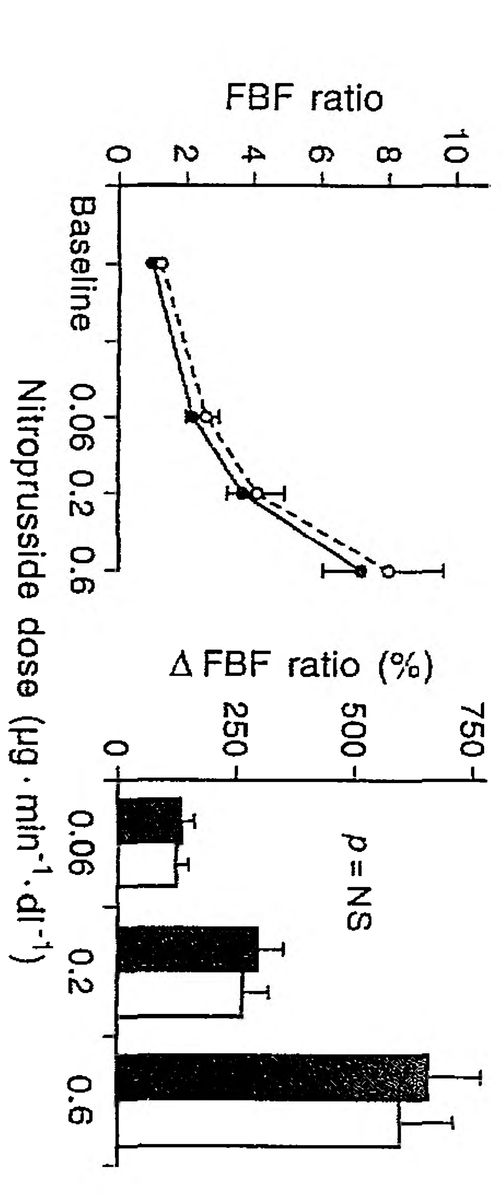


**Fig. 4.** The left panel shows mean (± SEM) absolute FBF ratios at baseline and during intraarterial infusion of the three sodium nitroprusside dosages (0.06, 0.2, 0.6) with concomitant intraarterial infusion of placebo (●), or with concomitant intraarterial infusion of low-dose glibenclamide (○). The right panel shows the corresponding mean (± SEM) percentage changes from baseline in FBF ratio. (■) Placebo; (□) glibenclamide. The $p$-value refers to ANOVA with repeated measures over the complete dose-response curves. NS, not significant

occurred during the highest diazoxide dose in this second part. These changes showed no differences between the protocols. Moreover, the FBF and the FVR in the non-experimental arm showed no statistically significant changes indicating that the systemic alterations in MAP and heart rate could not have contributed to the changes in FBF of the experimental arm.

*Changes in humoral parameters.* Low-dose glibenclamide did not affect systemic plasma glucose (from 4.6 ± 0.4 mmol/l before to 4.5 ± 0.2 mmol/l at the end of infusion), plasma insulin (from 117.4 ± 9.4 to 109.4 ± 7.9 pmol/l) or plasma C-peptide concentrations (from 0.79 ± 0.07 to 0.79 ± 0.03 pmol/l). However, the high glibenclamide dose significantly reduced plasma glucose from 4.7 ± 0.2 to 4.0 ± 0.2 mmol/l ($p < 0.001$), and raised plasma insulin and C-peptide concentration from 74.9 ± 15.1 pmol/l and 0.46 ± 0.05 nmol/l to 175.7 ± 28.8 pmol/l ($p < 0.01$) and 0.75 ± 0.09 nmol/l ($p < 0.01$), respectively. In the glimepiride series no significant changes in plasma concentrations of glucose (from 4.5 ± 0.1 to 4.5 ± 0.1 mmol/l), insulin (from 57.6 ± 8.6 to 45.4 ± 6.5 pmol/l) or C-peptide (from 0.55 ± 0.04 to 0.48 ± 0.04 nmol/l) occurred.

After 10 min of the single infusion of low-dose glibenclamide, the regional serum concentration averaged 193 ± 41 ng/ml, which fell to 48 ± 6 ng/ml during the highest vasodilator dose as a result of dilution ($n = 12$). At the end of the experiment the systemic glibenclamide concentration had accumulated to 17 ± 2 ng/ml. For the highest glibenclamide dose, the regional forearm concentrations at the end of the three diazoxide dosages were 881 ± 94, 508 ± 59 and 266 ± 32 ng/ml, respectively, whereas the systemic concentration at the end of the protocol averaged 152 ± 6 ng/ml. For glimepiride the local forearm concentrations were: 815 ± 97, 515 ± 72, 325 ± 25 ng/ml

after the three respective diazoxide dosages, whereas the systemic concentration averaged 194 ± 7 ng/ml at the end of the protocol.

## Discussion

The present study convincingly shows that the classical SU derivative glibenclamide is able to interact with vascular $K_{ATP}$ channel-activity in man. The intraarterial dosages of glibenclamide significantly inhibited the forearm vasodilator response to activation of $K_{ATP}$ channels by diazoxide. This attenuation could not be attributed to time effects since the repeat of diazoxide infusion resulted in an unaltered dilator response. The interaction between glibenclamide and diazoxide appeared to be of a specific character because glibenclamide did not attenuate the vasodilator response to SNP. Moreover, the new SU derivative glimepiride showed no interaction with diazoxide in our human in vivo model.

Intraarterial infusion of low-dose glibenclamide resulted in regional concentrations of ≈ 200 ng/ml, which even fell to ≈ 50 ng/ml during subsequent vasodilation as a result of dilution related to the higher flows, that agree with the systemic serum concentrations reached in NIDDM throughout the day after oral treatment with glibenclamide [20, 21].

Several in vitro studies in animal models have demonstrated that SU derivatives are able to block the vasodilator effects of a wide range of $K_{ATP}$ channel openers [3, 31, 32]. However, this does not necessarily hold for the human in vivo situation, especially because the high protein binding of glibenclamide may cause free concentrations to be too low to interact with cardiovascular $K_{ATP}$ channels [21, 33]. As such, we think that our observations are remarkable because they show for the first time that glibenclamide may interfere with vascular $K_{ATP}$ channels in man at clinically relevant concentrations.

The phenomenon of differences between the affinity of glibenclamide to $K_{ATP}$ channels in different tissues probably reflects the heterogeneity of these channels [34]. This implies that more selective agents should be able to block pancreatic $K_{ATP}$ channels sufficiently without interfering with cardiovascular $K_{ATP}$ channels. This selectivity could be more distinct because of the recently discovered different binding sites for SU derivatives [26, 35]. The newly developed SU derivative glimepiride has been shown to possess such a selectivity in animal experiments [15, 16, 26]. The present study shows that this selectivity is also true in the in vivo situation in humans, because in contrast to glibenclamide, the effects of the SU derivative glimepiride could not be distinguished from that of placebo. The absence of a vascular effect of glimepiride as opposed to glibenclamide cannot be attributed to a relatively low dosing for glimepiride, since intraarterial administration of glimepiride leads to local concentrations of 325 ± 25 ng/ml or higher, which are much higher concentrations than reached in the low-dose glibenclamide study, and are also higher than concentrations needed systemically to induce insulin production [23, 24].

Compared with the low glibenclamide dose, the 10 times higher dose of glibenclamide was not associated with a more pronounced block of diazoxide-induced vasodilation. The blockade by the low dose was far from complete; therefore, the higher dose was expected to further inhibit the response to diazoxide. This absence of a dose-response relationship may be explained by the fact that the high glibenclamide dose ultimately induced an increase in systemic insulin levels, although this small rise is not expected to induce effects on forearm blood flow [36]. Other possible explanations include: a maximal inhibitory effect already at the low glibenclamide concentration, the difference in study groups between the low- and high-doses and poor selectivity for the $K_{ATP}$ channel of the vasodilator diazoxide. However, none of these explanations are very likely [31, 37]. It is also unlikely that there is a delay because sulphonylurea derivatives have to diffuse into the cell membrane before reaching their receptor [38], since the results of the post-hoc tests showed a significant inhibition of the last diazoxide dose by the low-dose glibenclamide in contrast to the high-dose glibenclamide.

We did not find any effect of glibenclamide on the baseline FBF. This is in accordance with reports of a low open-state probability of the $K_{ATP}$ channel in vascular smooth muscle under baseline conditions [4].

Patients with NIDDM are relatively often exposed to myocardial ischaemia. During chronic treatment glibenclamide concentrations are close to those reached in the forearm in our study [1, 39], suggesting that at therapeutic concentrations glibenclamide has the potential to affect ischaemia-induced opening of cardiovascular $K_{ATP}$ channels. Blockade of $K_{ATP}$ channels by glibenclamide has been shown to impair $K_{ATP}$ channel-mediated protective effects [7, 14]. A recent study in man supports this view by showing that 10 mg of oral glibenclamide abolished ischaemic preconditioning during angioplasty [13]. Nonetheless it remains to be determined whether the acute effects of glibenclamide on pharmacological opening of vascular $K_{ATP}$ channels as observed in our study can be extrapolated to the chronic effects of glibenclamide on ischaemia-mediated opening of $K_{ATP}$ channels during treatment of NIDDM patients. Further it has to be emphasized that our results on the vascular effects of glibenclamide do not neceessarily hold for myocardial $K_{ATP}$ channels.

The closure of $K_{ATP}$ channels by SU derivatives has been shown to decrease the occurrence of ventricular fibrillation during or after ischaemia because it reduces the ischaemia-related $K^+$ efflux from the

myocardium [40, 41]. This dichotomous character of SU derivatives, with detrimental effects on contractile function and infarct size as well as beneficial effects on the incidence of fatal arrhythmias, may hamper conclusions from epidemiological studies as long as the relevant end points such as ventricular fibrillation and ejection fraction are not specifically included.

We conclude that the classical sulphonylurea derivative glibenclamide is able to specifically block vascular $K_{ATP}$ channels in humans at concentrations that occur in NIDDM patients using glibenclamide orally. In contrast, the newly developed sulphonylurea derivative glimepiride showed no evidence for this interaction. Because of the role of $K_{ATP}$ channel-opening in the pathophysiology of myocardial ischaemia and infarction, our observations warrant further studies on the clinical consequences of classic versus new sulphonylurea drugs in the treatment of NIDDM.

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